

CLAIMS

What is claimed is:

sub B47
1. A metal ion affinity peptide of a formula selected from the group consisting of:
formula 1: $(\text{His-X}_1\text{-X}_2)_{n1}\text{-(His-X}_3\text{-X}_4\text{-X}_5)_{n2}\text{-(His-X}_6)_{n3}$, wherein each of X_1 and X_2 is independently an amino acid with an aliphatic or an amide side chain, each of X_3 , X_4 , X_5 is independently an amino acid with a basic side chain (except His) or an acidic side chain, each X_6 is an amino acid with an aliphatic or an amide side chain, $n1$ and $n2$ are each independently 1-3, and $n3$ is 1-5;

formula 2: $(\text{His-Asn})_n$, where $n=3$ to 10; and

formula 3: $(\text{His-X}_1\text{-X}_2)_n$, wherein each of X_1 and X_2 is an amino acid having an acidic side chain, and $n=3$ to 10.

2. The metal ion affinity peptide according to claim 1, wherein the metal ion affinity peptide is of formula 1, and wherein each of X_1 and X_2 is independently selected from the group consisting of Leu, Ile, Val, Ala, Gly, Asn, and Gln.

3. The metal ion affinity peptide according to claim 1, wherein the metal ion affinity peptide is of formula 1, and wherein each of X_1 and X_2 is independently selected from the group consisting of Leu, Val, Asn, and Ile.

4. The metal ion affinity peptide according to claim 1, wherein the metal ion affinity peptide is of formula 1, and wherein each of X_3 , X_4 , X_5 is independently selected from the group consisting of Lys, Arg, Asp, and Glu.

5. The metal ion affinity peptide according to claim 1, wherein the metal ion affinity peptide is of formula 1, and wherein each of X_3 , X_4 , X_5 is independently selected from the group consisting of Lys and Glu.

6. The metal ion affinity peptide according to claim 1, wherein the metal ion affinity peptide is of formula 1, and wherein each X_6 is independently selected from the group consisting of Leu, Ile, Val, Ala, Gly, Asn, and Gln.

7. The metal ion affinity peptide according to claim 1, wherein the metal ion affinity peptide is of formula 1, and wherein each X_6 is independently selected from the group consisting of Ala and Asn.

Sub 1 8. The metal ion affinity peptide according to claim 1, wherein the metal ion affinity peptide is of formula 1, and wherein the affinity peptide has the amino acid sequence $\text{NH}_2\text{-His-Leu-Ile-His-Asn-Val-His-Lys-Glu-Glu-His-Ala-His-Ala-His-Asn-COOH}$.

9. The metal ion affinity peptide according to claim 1, wherein the metal ion affinity peptide is of formula 2, and wherein $n=6$.

Sub 2 10. The metal ion affinity peptide according to claim 1, wherein the metal ion affinity peptide is of formula 3, and wherein the affinity peptide comprises the sequence (His-Asp-Asp)₆.

11. The metal ion affinity peptide according to claim 1, wherein the metal ion affinity peptide is of formula 3, and wherein the affinity peptide comprises the sequence (His-Glu-Glu)₆.

12. The metal ion affinity peptide according to claim 1, wherein the metal ion affinity peptide is of formula 3, and wherein the affinity peptide comprises the sequence (His-Asp-Glu)₆.

13. The metal ion affinity peptide according to claim 1, wherein the metal ion affinity peptide is of formula 3, and wherein the affinity peptide comprises the sequence (His-Glu-Asp)₆.

14. A fusion protein comprising a polypeptide fused at its amino- or carboxy-terminus to a metal ion affinity peptide according to claim 1.

15. An isolated polynucleotide comprising a nucleotide sequence that encodes a metal ion affinity peptide according to claim 1.

16. The isolated polynucleotide according to claim 15, wherein the polynucleotide comprises a nucleotide sequence that encodes a fusion protein comprising a polypeptide fused at its amino- or carboxy-terminus to the metal ion affinity peptide.

17. A recombinant vector comprising a polynucleotide according to claim 15.

18. A recombinant host cell comprising a recombinant vector according to claim 17.

19. The recombinant host cell according to claim 18, wherein said cell is a prokaryotic cell.

20. The recombinant host cell according to claim 18, wherein said cell is a eukaryotic cell.

21. A method of purifying a fusion protein, the method comprising:

- a) contacting a sample comprising a fusion protein according to claim 14 with a metal ion chelate resin comprising a first metal ion; and
- b) eluting any resultant bound fusion protein from said resin.

22. The method of claim 21, wherein said resin comprises an immobilized Co^{2+} ion.

23. The method according to claim 21, further comprising:

- a) contacting the sample with a second immobilized metal ion affinity resin comprising a second immobilized metal ion; and
- b) eluting any resultant bound fusion protein from said first and second resins.

24. The method according to claim 23, wherein the first metal ion is a hard metal ion, and the second metal ion is an intermediate metal ion.

25. The method according to claim 24, wherein the hard metal ion is selected from the group consisting of Fe^{3+} , Ca^{2+} and Al^{3+} ; and the intermediate metal ion is selected from the group consisting of Cu^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} .

26. A kit for purifying a protein, comprising:

- a) a recombinant vector according to claim 17; and
- b) a metal ion affinity resin.

27. The kit according to claim 26, further comprising:

- an extraction buffer;
- a wash buffer; and
- an elution buffer.

28. The kit according to claim 27, further comprising a column.

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